WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 3, 166-175. Research Article ISSN 2277– 7105

TOTAL PHENOLIC CONTENTS AND FERRIC-REDUCING ANTIOXIDANT POWER FROM DIFFERENT PARTS OF MULTIPURPOSE TREE *(Moringa oleifera* Lam).

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Article Received on
05 Jan. 2019,

Revised on 26 Jan. 2019,
Accepted on 17 Feb. 2019

DOI: 10.20959/wjpr20193-14291

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ABSTRACT

Moringa oleifera played an important role in controlling oxidation caused by free radicals. Thus served as remedy to oxidative damages. This study was aimed to assess antioxidant activity and estimate total phenolic contents from different part of *Moringa oleifera* crude extract. Ferric-reducing antioxidant power assay was applied to evaluate antioxidant activity. Total phenolic compounds of the samples were measured using the Folin–Ciocalteu method. The reducing power of bark extract, revealed almost four times than any other part used, with (1499.67 ± 49.98 µM Fe (II)/g), followed by leaf with (432 ± 6.38 µM Fe (II)/g) and the lowest was seed extract having (126.67 ± 4.19 µM Fe (II)/g). The highest phenolic content was observed from methanolic bark (55.84 ± 2.92 mg GAE/g), followed by stem with bark extract having (48.81 ± 2.45 mg GAE/g), and the least was observed from stem extract with (42.25 ± 0.64 mg GAE/g). The result of this research showed that all the extract used are good source of natural antioxidant compounds. Therefore, *Moringa oleifera* can be utilized to cure oxidative related problems.

KEYWORD: *Moringa oleifera* Lam., antioxidant, phenolic, Ferric-reducing antioxidant power.

INTRODUCTION

Antioxidants are substances that prevent damage to cellwhich can be caused by free radicals that may eventually cause damage to DNA, leading to the possible development of cancer. The sources of free radicals could be related to UV light, ionizing radiation, cigarette smoke, pollutants, certain organic solvents, industrial waste and metabolism among others.[1,2] Added that oxidative damage causes many chronic diseases to human, examples of such diseases are cancer, atherosclerosis, diabetes mellitus, arthritis, ageing process, and neurode-generative disease. Some naturally anti-oxidant enzymes are synthesized inside the body naturally such as catalase and glutathione, while others can be derived from food sources for examples beta carotene, Vitamins A, E and C that are important antioxidants capable of regulating as well as eradicating free radicals from the body.[3] The major phenolic compounds in medicinal plant that are associated with antioxidant activities are flavonoids, phenols, tannins and alkaloids.[4] There are two types of anti-oxidant namely natural and synthetic anti-oxidant, however the earlier is safer to use than the later, and are highly bioactive compounds especially flavonoids and phenolics.[5] Natural antioxidants have a vital role in the inhibition of many age-related diseases and improving health.[6] It was also reported that carotenoid rich food are good protectants against certain diseases such as heart disease, cancer and diabetes.[7] It is known that some synthetic antioxidants such as butylhydroxytoluene and butylhydroxyanisole (BHT and BHA respectively) are highly toxic that can lead to health consequence, hence the replacement with natural anti-oxidant is required.[8]

The efficacy of *Moringa oleifera* as antioxidant was marked after the discovery of some natural antioxidants from the plant such as tocopherols, flavonoids,vitamin C and other phenolic compounds. Although[9] reported that epidemiological studies have proven that possibility of having cancer and coronary heart disease reduces through the intake of vitamin C.[6] It was also stated that moringa is a source of a number of bioactive compounds, specifically secondary metabolites e. g. alkaloids, tannins, phenolic compounds, phytosterols and terpenoids. Their main uses are anti-ulcer[10,11]; antipyretic[12]; anti convulsant[13]; anti-urolithiatic[14]; anti-inflammatory[15]; analgesic[16]; hepatoprotective activity against antitubercular drug-induced liver damage,[17] malnutrition[18,19]; anti-oxidant and hepatoprotective[20]; anti-malaria, and anti-diabetes[19,21]; anti-cancer,[18,22,23]; anti-hypertension, anti-hypoglycemia and anti-microbial activities, among others.[22,24] It wasreported the presence of some important compounds such asmoringinine and moringine.[21,25] However, many studies highlighted that *Moringa oleifera* is an outstanding

source of natural anti-oxidants which can served as a remedy to many diseases oxidative problems.

MATERIALS AND METHODS

Chemicals

Tripyridyltriazine, dimethylsulphoxide (DMSO), Folin-Ciocalteau, Gallic acid and Na_2CO_3 were used in this experiment, however, antioxidant activity of each part used were assessed through Ferric-reducing antioxidant power assays. Which were measured, via standard techniques of measuring antioxidant activity.

Plant material

Fresh leaves, stem and fruits of *Moringa oleifera* Lam. were collected from area of Terengganu, Malaysia. The plant were authenticated by the Faculty of Bioresources and Food Industry, Universiti Sultan Zainul Abidin, (UNISZA) Tembila, Terengganu, Malaysia. The plant was deposited at the University herbarium.

Extract preparation

The leaves, stem and fruit (pod) samples were washed properly and separated into stem (stalk), bark, stem with bark, pod and seed. Followed by subsequent dried at 40-43^{0}C. The dried samples were extracted with 100% methanol. The crude extracts were collected at least three times and were filtered through Whatman number 1 filter paper and then concentrated on rotary evaporator (Buchi, Flavil, Switzerland) at 45°C, dried and kept at 4°C till used for the assay. The extracts were dissolved in methanol to get the final concentration depending on the requirement.[26]

Total phenolic content assay

The total phenolic content of the samples was determined by adopting methods of Ainsworth (2007)[27] with some modification. Folin- Ciocalteu (F-C) reagent was used for the experiment, where 250µL of Methanolic extract was put in a test tube and mixed with 1.25ml of aqueous F-C reagent with 10% concentration, it is then incubated for 10 minute, it was then added with 1ml of 7.5% Na_2CO_3 solution, followed by second incubation for 30minute in dark before measurement at 650nm using spectrophotometer. Gallic acid solution served as a reference standard curve.

Ferric-reducing antioxidant power assay (FRAP)

The FRAP assay was carried out according to the modified method of Benzie and Strain (1996)[28] with a slightly modification. The FRAP reagent was prepared using 10 mmol TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mmol HCl, 20 mmol iron (III) chloride aqueous solution and acetate buffer (pH 3.6)in the ratio 1:1: 10 (v/v), respectively. FRAP reagent was prepared fresh for the experiment and was warmed to 37 °C in a water bath for 30 minutes prior to use. Fifty microliters of sample were added to 1.5 mL of the FRAP reagent. The absorbance of the reaction mixture was subsequently measured at 593 nm with spectrophotometer after 30 minutes of incubation. 2000 µM of the iron (II) sulfate solution was used as a standard and further diluted to 1000, 500, 250, 125, 62.5 and 31.25 µM, and the results were expressed as µmol Fe (II)/g dry weight of plant material. All of the measurements were taken in triplicate and the mean values were calculated.

Statistical analysis

Each assay was subjected to one way analysis of variance using statistical Package for Social Sciences (SPSS). significance level of 0.05% was used to test differences between the samples used.

RESULT AND DISCUSSION

Total phenolics

Total phenolic compounds of the samples were measured using the Folin–Ciocalteu method. Folin–Ciocalteu (F-C) reagent, a mixture of phosphomolybdic ($H_3PMo_{12}O_{40}$) and phosphotungstic ($H_3PW_{12}O_{40}$) acids, were reduced to blue oxides of molybdene (Mo_8O_{23}) and tungstene (W_8O_{23}) during phenol oxidation. This reaction takes place under alkaline conditions and sodium carbonate. The present of blue coloration occurs at 760 nm and simply indicate the quantity of phenols, it usually expressed as the chlorogenic acid or gallic acid equivalent.[29] In this experiment the total phenolic content from different part of *Moringa oleifera* was expressed in terms of gallic acid equivalence (GAE) of the extract. Total phenolic contents were calculated using the linear equation obtained from the calibration curve of gallic acid as follows

$$y = 0.0106x + 0.1027 \quad R^2 = 0.9991$$

The result revealed the range of phenolic content between 42.25 – 55.84, without much significant differences between the samples used. Where bark extract have significant phenolic content than any other samples used with an approximate value of 55.84 mg GAE/g,

followed by stem with bark, leaf and pods extract with 48.81, 45.90 and 44.89 mg GAE/g respectively, the significant diffence occur only between bark extract and stem, seed, pods and stem with bark at ($p > 0.05$), while the amount of total phenolic content found in stem was found to be very low compared to the rest of the samples used, having 42.25 mg GAE/g (Table 1). Generally these result indicates satisfactory phenolic content, the result corresponds with the result obtained by[30] where the highest total phenolic content found to be in a leaves extract, for samples from leaf, stem and stalk of *Moringa oleifera*. However, it was reported that most of the phenolic compounds such as flavonoids and their conjugates form a very large group of natural products. They are found in many plant tissues, where they are present inside the cells or on the surfaces of different plant organs.[31] Plant have being used tremendously as a source of phenolic compounds, especially beverages and vegetables, with an excessive amount that can help to reduce the risk or the level of many diseases, as a result of their antioxidant power.[32]

Table 1: Total phenolic content of methanolic extract from different part of *Moringa oleifera.*

Samples	TPC (mg/g GAE)
Leaf	45.9 ± 1.17^{ab}
Bark	55.84 ± 2.92^{a}
Pod	44.89 ± 4.82^{b}
Stem	42.25 ± 0.64^{b}
Stem with bark	48.81 ± 2.45^{ab}
Seed	44.77 ± 4.38^{b}

Values represent Mean SD of TPC (mg GAE/g) from different part of M*oringa oleifera* Lam, values with the same latter's were not significantly ($p > 0.05$).

Ferric-reducing antioxidant power assay (FRAP)

Absorbance of the control ferrous (II) sulphate revealed changes along the concentration gradient, thus the absorbance increases with the increase of concentration. The ability of different part of *Moringa oleifera* extract to reduce Fe^{3+} in to its Fe^{2+} was expressed as equivalence of ferrous sulphate (µM) and the result were calculated from the standard curve of Fe (II) sulphate, obtained via regression analysis. (Table 2), showed the FRAP values of different part from *Moringa oleifera* (Lam.), bark extract revealed higher FRAP values compare to the remaining part of the plant used with 1499.67 ± 49.98 (µM Fe (II)/g), followed by leaf, stem with bark and stem having 432 ± 6.38, 324.33 ± 4.11 and 216.33 ± 5.79 (µM Fe (II)/g) respectively, where is the least was observed from seed extract having

126.67 ± 4.19 (µM Fe (II)/g) (Table 2). The result correspond to the research done by [34] whom reported higher FRAP value from ethanolic Moringa leaf extract compared to fruits extract of the same plant, and the values were found to be lower than the current study. The FRAP value obtained by Nambiar *et al.,*[35] for the dried leaf extract of *Moringa oleifera* was found to be higher than the current research having 70.99mmol/100g of the dried leaf extract. Where are The FRAP value obtained by[36] from *Moringa oleifera* leaf was found to be lower than the current research. However[37] reported high FRAP values than the current experiment for the extract from ethanolic seed, pod, and aqueous extract of *Moringa oleifera* seed, nevertheless, current experiment was found to have higher FRAP values than ethanolic and aqueous extract of leaf, stem and pods of the same plant. The differences of FRAP values may be related to differences of solvent used for extraction, concentrations, standard compounds, geographical location of the plant and diluents used.

Table 2: Antioxidant capacities of different part of *Moringa oleifera* Lam. with respect to Ferric-reducing power assay.

Samples	Frap value (µM Fe (II)/g)
Seed	126.67 ± 4.19^{e}
Bark	1499.67 ± 49.98^{a}
Pod	195 ± 0.82^{de}
Stem	216.33 ± 5.79^{d}
Stem with bark	324.33 ± 4.11^{c}
Leaf	432 ± 6.38^{b}

Values represent Mean SD of FRAP (µM Fe (II)/g) from different part of M*oringa oleifera* Lam, values with the same latter's were not significantly ($p>0.05$).

CONCLUSION

Plant played an important role in controlling oxidation causes by free radicals. Thus served as a remedy to a number of oxidative damages. The result obtained from FRAP assay, and the presence of high phenolic compounds have revealed the potentiality of *Moringa oleifera* as a source of natural antioxidant, specipically from bark extract which shows high antioxidant. Hence there is need for researchers to further investigate possibilities of formulating a good herbal remedy from *Moringa oleifera* that can be used to prevent the progression of many diseases.

ACKNOWLEDGEMENT

My acknowledgement first and foremost is directed to Kano state Government for given

scholarship opportunity. through which this publication was accomplished, I would also acknowledge the effort of Mrs Rukiya Zainudin Biotechnology science officer, university Sultan Zainal Abidin, Malaysia for her wonderful assistance.

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